OPEN

Citation: Bone Research (2016) 4, 16050; doi:10.1038/boneres.2016.50
www.nature.com/boneres

REVIEW ARTICLE

Recent advances in nano scaffolds for bone repair

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Biomedical applications of nanomaterials are exponentially increasing every year due to analogy to various cell receptors, ligands, structural proteins, and genetic materials (that is, DNA). In bone tissue, nanoscale materials can provide scaffold for excellent tissue repair via mechanical stimulation, releasing of various loaded drugs and mediators, 3D scaffold for cell growth and differentiation of bone marrow stem cells to osteocytes. This review will therefore highlight recent advancements on tissue and nanoscale materials interaction.

Bone Research (2016) **4**, 16050; doi:10.1038/boneres.2016.50; published online: 13 December 2016

INTRODUCTION

Bone or osseous tissue is a significant and dynamic supporting connective tissue that continues to remodel and rebuild throughout the lifetime of an individual. Since bone is a scaffold of the body that is responsible for support, protection, locomotion and load bearing. In addition, it also undertakes responsibility for hematopoiesis, mineral homeostasis and other functions.

Currently, musculoskeletal maladies that result in tissue degeneration and inflammation are the main reasons for the disability and associated diseases around the globe.[1] In 2013, as reported in the Global Burden of Disease Study 2013 (GBD 2013) led by the Institute for Health Metrics and Evaluation (IHME), the burden caused by musculoskeletal maladies around the globe was 149 435 700 disability-adjusted life-years (DALYs), that mainly included rheumatoid arthritis, osteoarthritis, gout, low back and neck pain and other musculoskeletal disorders.[2] DALY is a unit to express the health losses from a type of disease and injury which computes the years of living with disability and years of life lost.[3] Even though bone tissue has internal repair and regeneration capacity, healing of large-scale bone defects caused by trauma, infection and tumor still needs external interventions.[4] There is, therefore, a huge demand for technologies and materials to ameliorate such kind of maladies.

Nanomaterials are synthetic or natural materials that have less than 100 nm size in either direction.[5] Technically, any material at nanoscale can be regarded as nanomaterial, but for better biomedical applications, the size should be in the range of 10–100 nm. Size above 100 nm may induce embolism and can be phagocytized and removed by the spleen, whereas reticulo-endothelial system and kidneys can readily clear the materials with size less than 10 nm.[6] Moreover, the size below 10 nm is more toxic and reactive due to higher surface density and increased surface reactive electrons. Nano-biomaterials are structural analogs to various body proteins, receptors, ligands and DNA (typically 5–20 nm size). This allows them to interact freely with various body receptors, easily crossing the cell membrane.[7] Nano-biomaterials are widely used in gene therapies,[8] nano-drug delivery systems,[9] cancer and various other disease theranostics,[10–14] sono and photo-dynamic therapies,[15] prosthetic orthopedic implants,[16] tissue engineering,[17] and so on. Nano-biomaterials combined with other medical methods may therefore have a key role in the near future.[18]

In this review, we mainly focus on the recent advances in nanomaterials application for bone tissue repair and prosthetic implants used to support the skeletal system. Conventional biomaterials that are used for bone tissue amelioration have been reported to have complications

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Received: 28 June 2016; Revised: 19 September 2016; Accepted: 30 September 2016

that result in elevated implant failure rate and delayed bone reparation. In this review, we will focus on the recent advances in nanotechnology for bone and supporting tissues reparation and amelioration.

BONE BIOLOGY AND REGENERATION

Macroscopically, bone tissue evolves into a variety of appearances to support different functions. To simplify the system, bones can be classified based on shape (that is, long, short, flat, and irregular bone), location (that is, axial and appendicular bone), or composition (that is, compact or spongy bone), and so on. Depending on their function, bones are only different in the pattern of arrangement, though they are all composed of same materials.

The bone matrix

Bone matrix consists of organic component, inorganic mineral component and water. Organic component accounts for approximately 25% of the weight of bone matrix, which includes type I collagen (~90%) and other non-collagenous proteins (for example, sialoprotein and osteopontin).[19] The non-collagenous proteins and proteoglycans account for a small total weight of organic component, though they still have an important role in osteoblast differentiation and tissue mineralization.[20] The mineral compartment of bone contributes to ~65% of the bone matrix by weight (primarily in the form of calcium hydroxyapatite (HA) –$Ca_{10}(PO_4)_6(OH)_2$). The bone microenvironment and nanocomposites biocompatibility are highly preferred when bone repair nanoscale materials are selected or designed.

Spongy bone and compact bone

There are two types of bone tissue present in most of the bones in the body; these are compact bone and spongy bone, representing 80% and 20% of the total bone mass, respectively.

Compact bone, is formed by cylindrical construction called Haversian or osteons systems and has an ordered histological pattern. The osteons run parallel to long bones and each of them contains lamellae that encircle a Harversian canal. Nerves and vessels go through the centric osteons canals whereas nutrients and waste products diffusion is limited. To exchange materials between the osteocytes and blood vessels, all the canaliculi build a branching network throughout compact bone. Based on the structural variations, bone regeneration materials should provide an adequate scaffold to support the autologous tissues, that is, shape and structure.

Progress in bone fracture healing

Bone tissue repair and regeneration is a dynamic process that starts with proliferation and migration of osteoprogenitor cells, finally realizing the reconstruction of bone with differentiation of osteoprogenitor cells and bone ECM formation. The scaffold materials which load different growth factors and drugs have achieved a great progress in bone tissue engineering.[21]

BMPs are members of a TGF-β family[22–23] that can significantly promote ossification in endochondral cells in mice after subcutaneous injection. Moreover, *in vivo* studies reported death of mice in early stages of development due to lack of BMP-2 or BMP-4.[24]

To date, even though the BMPs role in bone regeneration remains a challenge, some clinical studies give interesting clues. For instance, in fracture mouse model, the RNA levels of BMPs were tested during the course of damaged bone reparation. The results demonstrated that the BMP-2 and BMP-4 were expressed higher in early stages while BMP-5, BMP-6, and BMP-7 were expressed in terminal stages.[25] In humans, the expression of BMP-2, BMP-3, BMP-4, and BMP-7 represents regional differences in a callus tissue, BMP-3 and BMP-7 by higher expression in generation of new osteoblasts while BMP-2 and BMP-4 mainly exist in the mature bone tissue or hypertrophic chondrocytes.[26] All the clinical and pre-clinical researches support BMPs as an important factor in bone repair and clinical application.[27–28]

Wnt signaling molecules also have crucial role in regulating cell function, especially in osteogenesis and differentiation. Many Wnt proteins (for example, Wnt1, Wnt3a, Wnt4, Wnt5, Wnt10b and Wnt13) have key roles in regulating bone formation.[29] The Wnt proteins can promote proliferation of mesenchymal stem cell (MSCs) and their osteogenic differentiation; however, it can also inhibit the formation of cartilage cells and fat cells.[30] Zhong *et al.*[31] found in rat models that, Wnt signaling members expressed significantly, both at transcription level and protein synthesis after bone defect occurred. Chen *et al.*[32,33] found that fracture and β-catenin gene deletion in mice model can increase the proliferation of MSCs in the damage region, but they will differentiate to chondrocytes instead of osteoblasts, leading to the failure of bone repair. These results show that the Wnt/β-catenin signaling pathway is the core of mammalian bone biology and may provide new strategy for bone regeneration.[34]

The active factors that are loaded on scaffold materials, such as BMPs, Wnt, TGF-β, FGF, and VEGF can assemble the osteoprogenitor cells and induce them into specific cells, further regulating the regeneration of bone tissue and formation of ECM.[35] *In vivo* studies also confirmed that the growth factor could enhance the reparation of various fractures (that is, cannot heal itself after certain period).[36]

Requirements for materials in bone regeneration
inflammation

Any material that has been utilized in bone repairs and as prosthesis should be highly acceptable to the biological system, with less adverse effects. In case of bone regeneration, various factors are affecting the healing process either that influence this process independently or as co-factor with other multiple factors. Mentioned below are some of the most pivotal properties of nanoscale biomaterials and composites for bone repair.

Biocompatibility. A perfect bone repair scaffold materials should neither suppress the activity of normal cells nor toxicity during and after implantation.[37] In addition, it should also have osteogenesis-induced effects that may promote adhesion and proliferation of osteoblast or MSCs to form ECM. Cells can grow well in the three-dimensional (3D) microenvironment composed of nano-fibers. This is mainly because of a larger specific surface area that can promote adsorption of proteins, cell adhesion and growth.[38] Meanwhile, more and more nanomaterials are being synthesized with good biocompatibility for biomedical applications.[39–40]

Mechanical property. The bone repair scaffold should satisfy the mechanical strength and provide transfer properties. Mechanical strength of bone tissue from cancellous to density has a broad range. The differences between mechanical strength and geometrical mechanics in bone tissue make it difficult to design an ideal scaffold.[41] At the same time, various nanomaterials with good mechanical property are designed, such as nanofibers, nanopillars, nanoparticles, and nanocomposites,[42] which may help in this challenge.

Vesicular structure. The vesicular structure is a necessity for bone repair scaffold materials with porous diameter in at least 100 μm, to ensure the transportation of nutrients and oxygen.[43] It was found in one research study that scaffold materials with aperture size of 200–350 μm are best for bone tissue growth.[44] In addition, recent studies have shown that the scaffold with multiple aperture sizes have better repair effect than materials with only large aperture.[45] The bone repair materials have been successfully prepared by using polymer, ceramic, metal, and composite materials. Porous metal scaffolds can satisfy the mechanical requirements but cannot realize fusion with implants and tissues. Moreover, the metal ion will dissociate after implantation, which is also a serious problem that needs to be addressed.[46]

Bioabsorbability. Biological absorbability of the scaffold materials is another key factor for bone tissue regeneration.[37] An ideal scaffold material should be degraded *in vivo* at a certain time, with a controllable absorption rate that will finally provide a space for new bone generation. The degradation time for scaffold should also satisfy the application requirements, such that the materials used in spinal fusion need to be degraded after 9 months or longer, whereas materials in skull or maxillofacial bone should degrade in 3–6 months. The nanoscale scaffold materials are porous and biodegradable that can also provide mechanical support during the bone repair.[47]

Angiogenesis. An important requirement for bone repair materials is to promote the angiogenesis due to higher blood demands in the bone tissues.[43,48–50] The supply of oxygen and nutrients are indispensable for cells and tissues growth within the scaffold *in vivo*.[49] The inflammatory reaction for wound healing can induce spontaneous formation of blood vessels after scaffold implant.[43] It needs several weeks to form a vascular network; however, most scaffold materials do not have the ability to induce angiogenesis. In addition, incorrect or insufficient angiogenesis may hinder the delivery of oxygen and vital nutrients, which may result in uncontrolled differentiation or apoptosis of the cells.[51]

NANOMATERIALS APPLIED IN BONE REPAIR AND REGENERATION

The bone fracture, osteoporosis, osteoarthritis, and various neoplastic maladies are the most common clinical problems associated with bone and skeletal system. These common problems may be associated with malnutrition, aging, hormonal imbalance or trauma. It is estimated that around 2.2 million bone tissue graft transplants are performed all around the globe annually.[52] The autograft is most common orthopedic implant but has certain well-documented limitations (that is, resorption, donor site morbidity, compromised supply, and rejection rate of up to 50% at some sites[53–54]). Mostly, the complicated and multiple fractures due to trauma or age (mostly at hip joint, that is, femur head fractures) are supported with prosthetic implants for proper healing. These implants are comprised of various materials known as biomaterials. Nevertheless, after 10–15 years on average, the traditional implant failure is associated with biomaterial associated inflammation, loosening, wear or tear debris, osteolysis and autoimmune reactions.[55] These snags urge for the development of biomaterials with greater cytocompatibility and long lasting life, with higher patient's quality of life. The role of nanotechnology and nanomaterials therefore becomes very pivotal. Various nanocomposites, materials and particles have been applied to mimic the growth of bone

tissues, lower the autoimmune reactions and keep check on microbial infections.[56–59] Herein, we also mainly focus on the nanomaterials role in bone tissue repair, support, and maintenance.

Influence on bone regeneration

Organic bone tissue has various protein (collagen, fibronectin, laminin, and vitronectin) and water as soft hydrogel nanocomposites, whereas HA and Ca_{10} $(PO_4)_6$ $(OH)_2$ are hard inorganic components for the bone.[60] The HA is present in nanocrystal line form which is 20–80 nm long and 2–5 nm thick, whereas the other proteins in the ECM are also at nanoscale size. This structural analogy allows the nanomaterials to interact easily with bone tissue and influence its functionality. Among the proposed nanoscaffolds for bone regeneration, Cerium (Ce-HA)[61] based structures are among the leading candidates for bone tissue engineering. Similarly, a Mg-HA/collagen type I scaffold may also have great utility in bone regeneration.[62] Besides, nano-HA together with chitosan (CS), Jiang *et al*.[63] reported sodium carboxymethyl cellulose (CMC) hybrid membrane that was curled in a concentric manner to realize an anisotropic spiral–cylindrical scaffold. The cylinder-shaped scaffold has similarity to natural bone expedited complete infiltration of bone tissues *in vivo* and finally realized osteointegration and functional reconstruction of damage bone, as shown in Figure 1a.

Materials, at nanoscale, have been reported with better cell functionality than micro or macro scaled materials.[64] The ECM provides scaffolds for the growth, proliferation and influence functionality of various cells. The nanoscale materials mimic the intrinsic and extrinsic pathways of osteocyte differentiation and mobility. Cells in various parts of the body exist in either two-dimensional (2D) or 3D environment, for example, stem cells in the intestinal crypts exists in 2D environment, whereas stem cells in bone marrow exist in 3D environment.[65] The nanomaterials may provide the desired environment for the proliferation of various cells in a bone niche. Similarly, the magnetic nanoparticles in addition to influence on osteocytes intrinsic pathways, may also act as mechanical stimulus that will help in the healing process.[66] The silk fibroin-hydroxybutyl chitosan blended nanofibers successfully provided scaffold for the growth of porcine iliac endothelial cells. The nanofibers provided typical ECM to cells, where these cells formed endothelial monolayer with higher confluency.[67] In Figure 1b, Wang *et al*.[68] developed apatite-collagen-polycaprolactone (Ap-Col-PCL) composites that showed excellent bioactivity to promote fast bone regeneration in rabbit model with fractional long bone defect. They combined rapid prototyping (RP) fabrication technology and 3D functionalization strategy for biomimetic deposition and collagen incorporation. These composite materials showed outstanding mechanical properties similar to cancellous bone, good biodegradability, and hierarchical architecture of three nano–micro–macro levels.[68]

Bioactive materials

Bioactivity is the ability for a material to mimic response in living system.[69] The orthopedic bioactive materials should elicit the biological response at interface and build a strong bond between the material and bone tissue.[70] Hence, the role of bioactivity is inevitable for biomedical applications of biomaterials. The bioactive materials for bone repair are mainly divided into osteoconductive and osteoproductive, depending upon the rate of implant and its tissue interaction.[71] The bioactive materials are mainly fabricated by either tailoring of bioactive composites and coatings or molecular surface tailoring. The later one is ideal for bone growth promoting factors, that is, BMPs. They are considered most important factors for the proliferation and growth of the bone tissue.[72] The nanoarrays of gold has immobilizing effect on BMP-2, which allows the controlled release of BMP-2 that may have important role during the bone tissue repair via osteoblasts.[73–74] The BMP-2 signals, differentiation and proliferation were also found to be significantly increased after treating cells with ceramic conjugated nanoparticles.[75] Nanofibrous membranes (NFMs), for instance, with BMP-2 in the core and silk fibroin/chitosan/Nanohydroxyapatite (SCH) as the shell, were developed and tested both *in vitro* and *in vivo* for modulation of bone regeneration, results also suggesting the NFMs as an excellent scaffold for bone tissue engineering.[76] (Figure 2a) Similarly, collagen-containing hydrogel was seeded with magnetic nanoparticles to target TWIK-related K(+) channel (TREK)-1 for enhanced mineralization on experimental basis. Moreover, the bone mineralization was significantly increased by mechanotransduction.[77]

Influence on bone tissue cells and BMSCs. In osteogenesis and bone mineralization studies, various biochemical mediators, including ascorbic acid, dexamethasone, BMPs, and β-glycerophosphate, are supplemented to differentiating medium or incorporated in biomaterials. However, some studies reported nanoscale composites with osteoinductive effect without addition of any biochemical mediator, suggesting strong influence of dimensional structure niche and cell's shape.[78] Notably, Khanna *et al*.[79] reported chitosan-polygalactouronic acid hydroxyapatite (Chit-Pga-HA) nanofibers with osteoconductive and osteoinductive properties by mimicking the natural bone mineralization and collagen formation. Similarly,

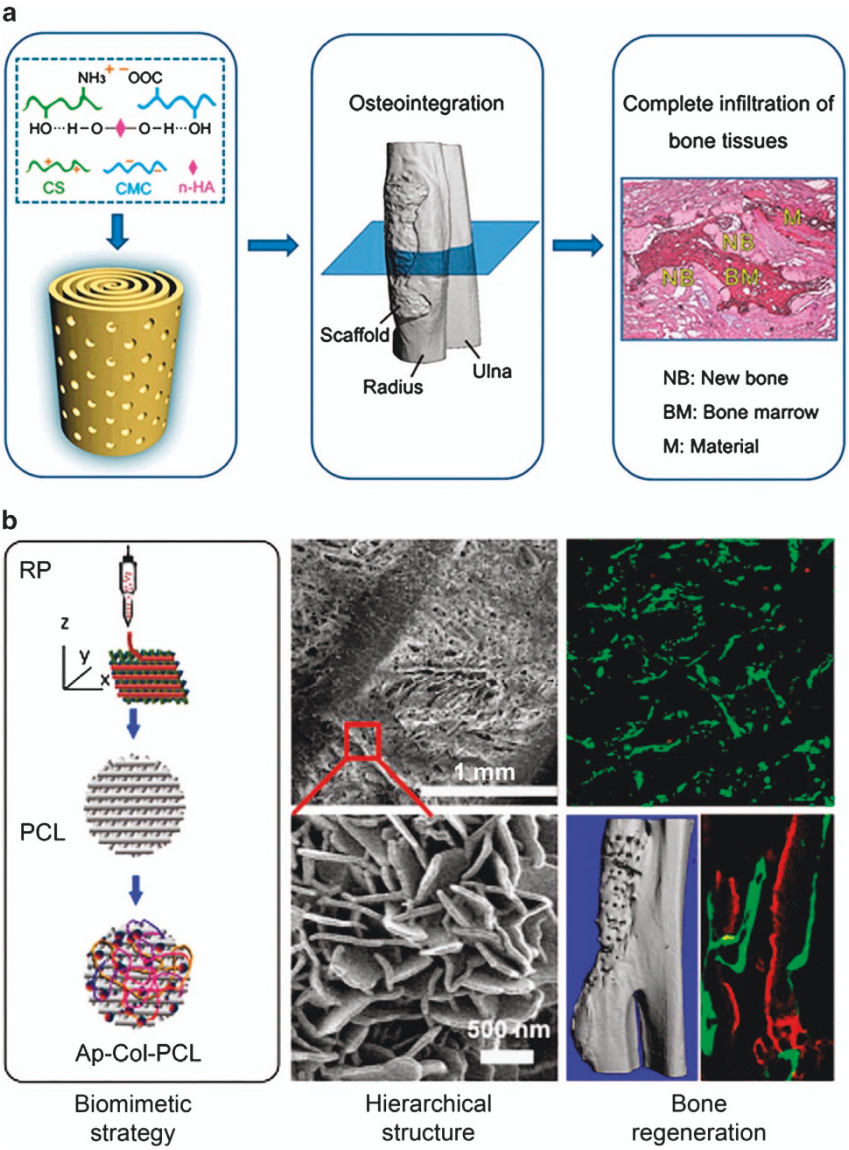

Figure 1. (**a**) Biomimetic spiral-cylindrical scaffold based on hybrid chitosan/cellulose/nano-hydroxyapatite membrane. (**b**) Biomimetically ornamented rapid prototyping fabrication of an apatite – collagen – polycaprolactone composite construct with nano – micro – macro hierarchical structure. Reprinted with permission from ref. 63 2013 ACS Publishing Group and ref. 68 2015 ACS Publishing Group.

Roohani-Esfahani *et al.*[80] coated biphasic calcium phosphate struts with bioactive glass nanoparticles and found 14 times increase in compressive strength and enhanced differentiation of primary human derived bone cells by upregulating the Runx2, osteopontin and sialoprotein genes. Moreover, the recent findings by Tutak *et al.*[81] suggested that poly (ε-caprolactone) (PCL) nanofibers promoted the differentiation of human osteoprogenitor cells by changing the organelle structure and positioning, which resulted in altered cells functionality. Besides, as reported by Tang *et al.*,[82] recombinant human bone morphogenetic protein-2 (rhBMP-2) was loaded on a trimodal (macro/micro/nano) mesoporous bioactive glass scaffold (TMS) with enhanced compressive strength. They tailored a 7.5 nm, 3D cubic mesoporous structure for a "size-matched entrapment" of rhBMP-2, so the TMS/rhBMP-2 could achieve sustained release and appealing bone regeneration capacity (Figure 2b).

The nanostructure arrays of various biomaterials (for example, polymethylmethacrylate (PMMA) 120 nm pit and 100 nm diameter size with 300 nm interspace) have been reported with efficient osteogenic differentiation of bone marrow mesenchymal stem cells BMSCs.[83] Similarly, Tarpani *et al.*[84] used 130 nm silica (SiO_2) nanoparticles functionalized by amino group (SiO_2-N) and silver (SiO_2-Ag) nanoparticles for the growth of human BMSCs

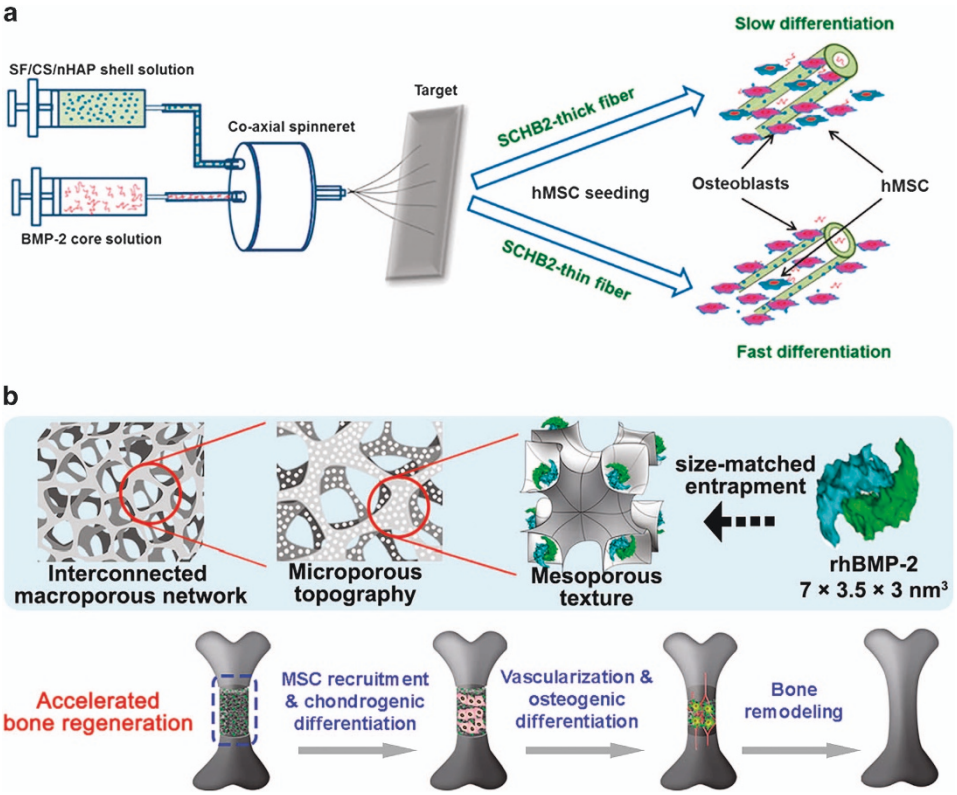

Figure 2. (**a**) Preparation of SCHB2-thick and SCHB2-thin NFMs through coaxial electrospinning and their influence on hMSCs. (**b**) Tri-modal macro/micro/nano-porous scaffold loaded with rhBMP-2 for accelerated bone regeneration. Reprinted with permission from ref. 76 2015 ACS Publishing Group and ref. 82 2016 Elsevier Publishing Group.

and observed good interaction between the silica nanoparticles and BMSCs, making it a strong candidate for future bone tissue engineering. The recent findings by Rehman *et al.*[85] also suggested strong proliferating effect of TiO_2 nanowhiskers and tetra sulphonatophenyl porphyrin (TSPP) nanocomposites on rheumatoid arthritis BMSCs. In addition, Zuyaun *et al.*[86] used the mesoporous silica based nanocomposites loaded with BMP-7 to differentiate the BMSC from osteocytes by slowly and constantly releasing the BMP-7 as trigger of the osteogenesis. Xia *et al.*[87] reported the highly interconnected microporous HA bio ceramic scaffolds whose surface was modified by nanosheet, nanorod and micro-nano-hybrids. The materials not only promoted cell attachment, proliferation, spreading and osteogenic differentiation of adipose derived stem cells (ASCs), but also enhanced the expression of angiogenic factors. The combination of the HA scaffolds with nano-surface and ASCs could enhance both osteogenesis and angiogenesis in a rat critical-sized calvarial defect model.[87]

Extra cellular matrix and bone supporting tissues. The bone tissue is a part of complex skeletal system that is also comprised of various tissues (for example, ligaments and tendons). The attached ligaments and tendons after trauma may also need regeneration; hence, the nanotechnology may be applied for enhancing strength and biocompatibility. Recently, Sheikh *et al.*[88] incorporated multi-walled carbon nanotubes into polymeric nanofibers to form ideal candidate for bone tendon and ligament repair after trauma. They reported that addition of MWCNTs to the polymeric nanofibers increased the tensile strength from 11.40 ± 0.9 to 51.25 ± 5.5 Mpa.[88] Moreover, the fibroblast cells attachment and higher viability rate indicated the biocompatibility of the said artificial ligaments/tendon candidate. The ligament advanced reinforcement system (LARS) is also considered a promising graft when nanomaterials, such as nano-silica, are applied to its surface; both the biocompatibility and ligament reconstruction effectiveness of LARS are improved.[89] Moreover, some other studies reported the co-electrospun scaffold which was based on nano-hydroxyapatite particles, as well as Medtronic's recombinant, could up-regulate the expression of BMP-2 and osteopontin on mineral-containing region, and may promote the regeneration of the ligament-bone interface.[90–91]

Composite materials

Various synthesized hydrogels are good for providing extra cellular matrix for proliferation of cells during the healing process. The higher water content provides cells friendly microenvironment for performing various functions. Mostly during bone fracture the vasculature is compromised, which can be mimicked by various factors. The use of composite materials may allow the angiogenesis without any vital biochemical factor. The recent findings by Mammadov *et al.*[92] suggest that the use of polymers to mimic angiogenesis without any soluble factor is a new approach in tissue regeneration. The same technique may be used for bone regeneration, especially in complex fractures where the vasculature is compromised.[92] Other composite nanomaterials, such as Ca^{2+}-induced Bombyx mori silk sericin (BS)/HA, reduced graphene oxide (rGO)/HA and recombinant human vascular endothelial growth factor (rhVEGF)/nano-HA/coralline blocks could also significantly promote the proliferation and osteogenic differentiation of the BMSCs for bone repair.[93–95] Zhao *et al.*[96] recently used tetra sulphonatophenyl porphyrin derivatives adjuvant with TiO_2 nanowhiskers for theranostics of Rheumatoid Arthritis. These nanocomposites were not only biocompatible but also had protective effect on the synovial milieu and long bones tissue.[96]

Similarly, nano-TiO_2 has been used for coating of orthopedic prosthetic implants.[97] The TiO_2 nanotubes have been used in the articular joints, that is, hip and knee joint, to minimize the wear and tear effect; however, it was not very successful due to inflammatory reactions. The nanoscale TiO_2 particles coated on the surface of prosthetic implants are safer with enhanced bone mineralization and osteoblast adhesion.[98–99] Earlier studies reported that orthopedic prosthetic implants coated with TiO_2 nanotubes were successfully loaded with non-steroidal anti-inflammatory drugs (for example, Ibuprofen[100]) and variety of antibiotics and antibacterial (for example, gentamycin[101] and cefuroxime[102]) to keep check on infection and inflammation, without compromising the adhesion of osteoblasts to the implanted biomaterial.

Bone tissue requires dynamic mechanical stimulation for its proper functionality. In nanotherapeutics, it can be fulfilled by various magnetic nanoparticles that upon exposure to magnetic field may alter the cells physiological and biochemical environment by moving the charged particles into the cell by enhanced membrane permeability.[64]

Nano-coating of implants

Nanoscale structures and coating of various prosthetic implants is of higher interest in orthopedic surgery due to lower debris generation, especially in articular joints. The prosthesis main body is comprised of metallic alloy (that is, Ti-6AL-4 V, cobalt-chromium-molybdenum) which articulates against polymer or ceramic-polymer surface (alumina, aluminia-zirconia, ultra-high molecular weight PE). The excellent tribo-corosion and biocompatibility can be achieved via surface coating with nanotubes, nano-whiskers, diamond, and graphite like carbon, titanium,[103] and tantalum.[104] Along with anti-friction coating, the nanobiomaterials are also favored for control of infections by loading various antimicrobials on prosthesis surface. The nano-titania and silver particles coating on the prosthetic implants are very extensively used in orthopedic prosthetic implants to control post-operative complications and infections. Recently, Singh *et al.*[105] prepared 25–35 nm HA coated on the Ti-alloy to lower the graft-versus-host disease (GVHD) to orthopedic implants and increased its biocompatibility. Stanic *et al.* synthesized Silver (Ag_2O) fluroappatite nanopowder with 80 nm average length and 20 nm width, finding excellent antibacterial effect on klebsiella pneumoniae, Staphylococcus aureus and Micrococcus luteus due to the antibacterial effect of sliver, which can be potentially explored in orthopedic implants.[106] Another biomimetic HA nano-construct was synthesized by Koirala *et al.*,[107] which could modify a Ti implant. The nano-HA covered with a phospholipid bilayer may support long-term sustainability of implants. Although, the nanomaterials used in bone implants are also having adverse effects on the bone cells, for example, the silver (Ag) nanoparticles (80 nm) and ions are reported with delayed differentiation of human MSCs to osteocytes and adipocytes, even at biocompatible concentration.[108]

3D technology

In early eighties of twentieth century, Charles Hull was the first to report 3D technology for printing various objects. Afterwards, applications of the 3D technology got momentum in various fields, including biomedicine, for tissue regeneration and transplant, especially bone. Among various major concerns in the 3D technology, the bioresorption and biocompatibility are major issues. Most of the important bone materials used in bone 3D printing include calcium phosphate ceramics and cements, HA, brushite, monetite, β-tricalcium phosphate (TCP) and bioactive glass mixture, due to their comparable analogy to bone minerals and higher biocompatibility.[109–110] Porosity of the implant is pivotal for the growth and attachment of bone tissue to implant. The ideal porosity has been reported with 30%–70% prosthetic comprised of 500–1000 μm, respectively.[111] However, the bone tissue is comprised of various nano (collagen– I and

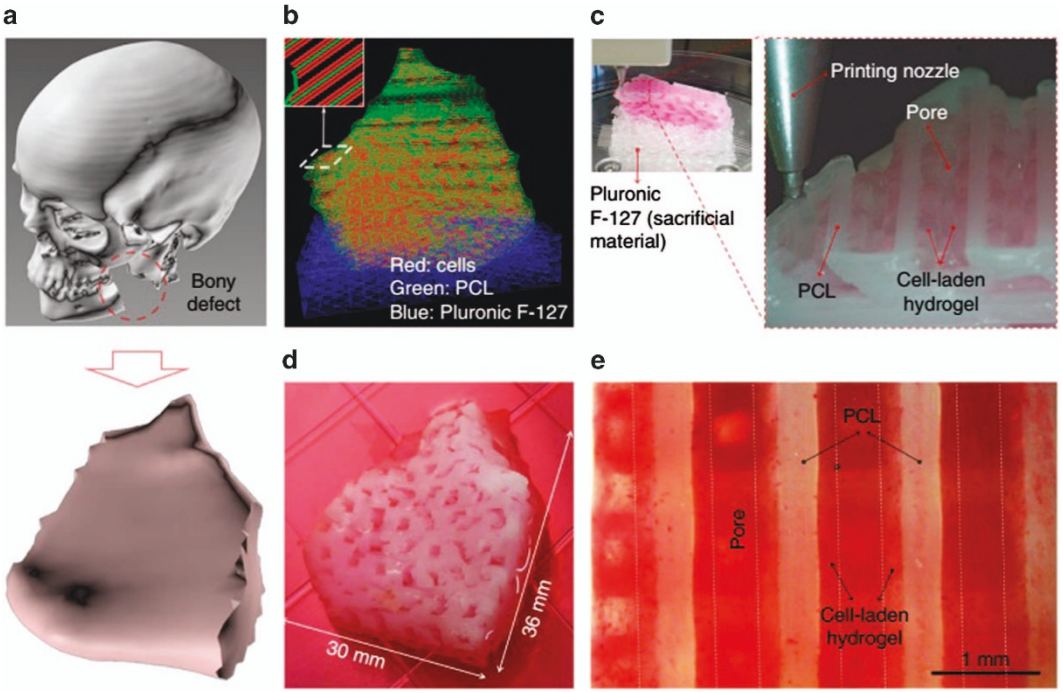

Figure 3. Mandible bone reconstruction. (**a**) 3D CAD model recognized a mandible bony defect from human CT image data. (**b**) Visualized motion program was generated to construct a 3D architecture of the mandible bone defect using CAM software. Lines of green, blue and red colors indicate the dispensing paths of PCL, Pluronic F127 and cell-laden hydrogel, respectively. (**c**) 3D printing process using integrated organ printing system. (**d**) Photograph of the 3D printed mandible bone defect construct, which was cultured in osteogenic medium for 28 days. (**e**) Osteogenic differentiation of hAFSCs in the printed construct was confirmed by Alizarin Red S staining, indicating calcium deposition. Reprinted with permission from ref. 112 2016 Nature Publishing Group.

other proteins) to macro structures, and subtle disturbance or disorientation in these structures will lead to maladies (for example, osteogenesis imperfecta and brittle bone disease). Recently, Kang *et al.*[112] demonstrated a mandible bone reconstruction using human amniotic fluid–derived stem cell (hAFSC)-laden hydrogel, a mixture of PCL and TCP, and Pluronic F127 (Figure 3b). The PCL/TCP and hAFSCs mixed with the composite hydrogel were printed in a type I pattern with a Pluronic F127 temporary support (Figure 3c). After induction of osteogenic differentiation for 28 days (Figure 3d), they stained the structures with Alizarin Red S; staining at the surface of the 3D bone structures indicated calcium deposition in the hAFSC-laden hydrogel (Figure 3e).

The HA based nanocomposites are favored for the nano 3D structure formation due to promotion of cell organization, proliferation and allowance of free movement of nutrients to the developing tissues. The recent findings by Jun *et al.* suggest that sphere shaped nano-HA-chitosan-gelatin based scaffolds accelerated the fibroblast iPSC (induced pluripotent stem cells) osteogenesis as compared with rod shaped nano-HA-chitosan-gelatin scaffold both *in vivo* and *in vitro*.[113]

CONCLUSION

In summary, biomedical applications of the nanoscale materials in amelioration and regeneration of skeletal system, especially in bone and supporting tissues, are highly appreciated in modern therapeutics and surgery. The 3D scaffold, structural analogy, biocompatibility, growth promoting properties, and time-bound degradability of nanoscale materials make them ideal candidates for orthopedic prosthetic surgeries and bone reparation.

Acknowledgements

This work was funded by the 863 project (2015AA020502), National Natural Science Foundation of China (61401217, 61527806), Natural Science Foundation of Jiangsu Province (BK20140900), and the Economical Forest Cultivation and Utilization of 2011 Collaborative Innovation Center in Hunan Province [(2013) 448].

Competing interests

The authors declare no conflict of interest.

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